U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

Jorge Manuel Ramos das Neves da Silva

Eosinophils in the gastrointestinal tract: how much is normal? / Eosinófilos no tracto gastrointestinal: quantos são considerados normais?

março, 2018

Jorge Manuel Ramos das Neves da Silva

Eosinophils in the gastrointestinal tract: how much is normal? / Eosinófilos no tracto gastrointestinal: quantos são considerados normais?

Mestrado Integrado em Medicina

Áreas: Anatomia Patológica e Pediatria

Trabalho efetuado sob a Orientação de:
Doutora Maria de Fátima Machado Henriques Carneiro
E sob a Coorientação de:
Doutor Jorge Manuel Bastos Amil Dias

Trabalho organizado de acordo com as normas da revista:
Journal of Pediatric Gastroenterology and Nutrition

março, 2018

U.PORTO
FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Jorge Manuel Ramos dos Novos da Silva, abaixo assinado, nº mecanográfico 201205452, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 19/03/2018

Assinatura conforme cartão de identificação:

Jorge Manuel Ramos Novos da Silva

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

JORGE MANUEL RAMOS DAS NEVES DA SILVA

NÚMERO DE ESTUDANTE	E-MAIL
201205452	jorge.ramos.neves.silva@gmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

ANATOMIA PATOLÓGICA E PEDIATRIA

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Eosinophils in the gastrointestinal tract: how much is normal?

ORIENTADOR

Prof. Dra. MARIA DE FÁTIMA MACHADO HENRIQUES CARNEIRO

COORIENTADOR (se aplicável)

Dr. JORGE MANUEL BASTOS AMIL DIAS

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☒

Faculdade de Medicina da Universidade do Porto, 19/03/2018

Assinatura conforme cartão de identificação: Jorge Manuel Ramos Neves da Silva

Dedicatória

Dedico, com o maior afeto, este trabalho aos meus pais, Ana e Jorge, à minha irmã, Ana, e à Marta, incansáveis no alento e os mais sábios conselheiros, nesta derradeira travessia.

Dedico também aos meus avós, padrinho e madrinha, tios e tias, primos e primas que me acompanham desde sempre.

Eosinophils in the gastrointestinal tract: how much is normal?

Authors:

Mr. Jorge **Silva**, BSc, Medical School of Porto, Portugal

Mr. Pedro **Canão,** MD, Centro Hospitalar S.João, Department of Pathology, Porto, Portugal

Mrs. Maria Céu **Espinheiro,** MD, Centro Hospitalar S.João, Department of Paediatrics, Porto, Portugal

Mrs. Eunice **Trindade,** MD, Centro Hospitalar S.João, Department of Paediatrics, Porto, Portugal

Mrs. Fátima **Carneiro,** MD, PhD, Centro Hospitalar S.João, Department of Pathology, Porto, Portugal; Medical School of Porto, Department of Pathology, Portugal; Ipatimup, Porto, Portugal

Mr. Jorge Amil **Dias,** MD, Centro Hospitalar S.João, Department of Paediatrics, Porto, Portugal

Correspondence:

Jorge Silva, BSc, Medical School of Porto, Portugal

Address: Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

Telephone number: +351969273257

Email: jorge.ramos.neves.silva@gmail.com

Word count: 3127

Number of tables: 3

Number of figures: 2

Conflicts of Interest and Source of Funding:

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article and that they have no financial support from any institution.

Acknowledgments

The authors would like to acknowledge Mrs. Marta Rodrigues, BSc, for her significant help with the haematoxylin and eosin stained slides and complete availability.

Authorship:

Mr. Jorge **Silva**: Conception and design of the work; analysis (eosinophil counting's and statistics) and interpretation of data (results and discussion); review of bibliography; drafting of the work and review of the intellectual content; final approval of the version to be published.

Mr. Pedro **Canão:** Design of the work; analysis of data (eosinophil counting's); review of the intellectual content; final approval of the version to be published.

Mrs. Maria Céu **Espinheiro:** Design of the work; analysis of data **(**review of the endoscopic reports to verify absence of mucosal disease); review of the intellectual content; final approval of the version to be published.

Mrs. Eunice **Trindade:** Design of the work; analysis of data (review of the endoscopic reports to verify absence of mucosal disease); review of the intellectual content; final approval of the version to be published.

Mrs. Fátima **Carneiro:** Design of the work; analysis (selection of the patients for the study; review of the final pathological reports and reassessment of the slides to exclude histological abnormality) and interpretation of data (results); review of the intellectual content; final approval of the version to be published.

Mr. Jorge Amil **Dias:** Design of the work, analysis (review of the endoscopic reports to verify absence of mucosal disease; criteria for inclusion of the patients after primary selection); and interpretation of data (results and discussion); review of the intellectual content and scientific misspelling; final approval of the version to be published.

ABSTRACT

Objectives: The normal density of eosinophils in the digestive mucosa of children has been rarely addressed despite being important to provide baseline counts for the diagnosis of eosinophilic gastrointestinal disorders (EGID). Even though histopathological criteria for EGID remains undefined, there has been little consistency of results in different populations. We aimed to establish the eosinophil density of the normal digestive mucosa in a paediatric population submitted to endoscopic procedures that were reported as normal.

Methods: Biopsies from endoscopies of 33 patients were evaluated. Quantification of eosinophils was performed manually. Review of the pathology reports confirmed absence of abnormality in the biopsy specimens. Counts were reported as mean±standard deviation eosinophils per mm^2.

Results: *Oesophagus* (n=33): eosinophils were uniformly absent in all biopsies. *Stomach*: fundus (n=14; 0.7±0.9), body (n=15; 0.3±0.6) and antrum (n=18; 0.6±1.5) revealed consistent values in the *lamina propria*. *Small intestine*: eosinophil counts revealed 17.8±16.6, 14.2±11.8, and 50.4±34.6 in the *lamina propria* of the bulb (n=13), second segment of duodenum (n=13) and ileum (n=16), respectively. *Large intestine*: the highest peak count was observed in the caecum (123; n=16) with a mean of 50.8±32.8. The eosinophil counts were lower in the ascending (n=16; 40.2±26.8), transverse (n=14; 33.6±21.5), descending (n=15; 39.2±26.1) and sigmoid (n=17; 25.3±17.4) colon and in the rectum (n=17; 13.6±9.9). Eosinophils were regularly absent in the surface epithelium or/and crypt epithelium in these segments.

Conclusions: These data provide a baseline count and distribution of eosinophils in the gastrointestinal tract of paediatric patients with normal histology, thus expanding the scarce published data.

Keywords: eosinophil, gastrointestinal tract, normal, endoscopy, histology.

What is known?

Eosinophils normally reside in the gastrointestinal tract (GI), with different density in each segment. There is little information about the normal range of eosinophils in the GI tract of children. Different populations may have specific prevalence of eosinophils in the GI tract due to allergy or parasitosis therefore comparison and validation of normal range is needed.

What is new?

A detailed analysis of each segment of the GI tract and different layers of the mucosa led to calculation of normal density of eosinophils in normal biopsies. This study expands the scarce published data and provides contribution to evaluate children with suspected eosinophilic gastrointestinal disorders.

INTRODUCTION

The importance of eosinophilic gastrointestinal disorders (EGID) has been increasing over the past two decades[1–3]. Collins *et al*[4] defined EGID as diseases that characteristically exhibit excessive numbers of eosinophils, in normal and abnormal locations, in one or more gastrointestinal (GI) segments. Eosinophils normally reside in the GI tract[5], so its mere presence does not postulate a diagnosis of EGID. In contrast, these cells are normally absent in the oesophagus. The presence of 15 eosinophils per high power field (HPF), accepted as the minimum number required for the diagnosis of Eosinophilic Oesophagitis (EoE), has being used to define EoE histologically[6]. Other forms of EGID remain with undefined histologic criteria. This illustrates the need to further evaluate the normal density of eosinophils in the digestive mucosa. Until now, only a few studies[7–12] aimed at defining reference values. This is important to provide baseline counts as a reference for the diagnosis of EGID. Given the small number of studies, the consistency of results in different populations was never fully evaluated.

We aimed to establish the eosinophil density of the normal digestive mucosa in a paediatric population submitted to endoscopic procedures that were reported as normal. We evaluated endoscopic biopsies from each segment of the GI tract of paediatric patients without organic pathology based on histological and endoscopic reports. These data provide a baseline count and distribution of eosinophils in the GI tract of paediatric patients with normal histology and provide an additional contribution to evaluate children with suspected EGID.

METHODS

Enrolment:

The paediatric population included in this study was retrospectively identified and randomly selected from the hospital database (SClinico). It consisted of patients that underwent endoscopic procedures in the diagnostic process for suspected disease. To

be eligible: (1) the final pathology report had to be normal in all evaluated segments and (2) the final clinical diagnosis could not involve organic GI disease associated with abnormal density of eosinophils (for example: inflammatory bowel disease). Based on these criteria, 33 patients were selected for this analysis. Patients diagnosed with functional GI disorders (FGID), namely Irritable Bowel Syndrome (IBS), were not excluded but analysed separately as defined below. The absence of histological abnormality was confirmed by review of the final pathology report and reassessment of the slides by a senior pathologist (F.C). Coeliac disease (CD) was excluded by review of the pathologic and serologic data recorded at the time of the clinical work-up. Moreover, the endoscopy reports were evaluated to verify the absence of mucosal disease at the time of the endoscopy. Importantly, all gastric biopsies, from the selected patients, were free of *Helicobacter pylori* organisms and no parasites were detected in all evaluated biopsies.

In the first instance, all biopsies were included to access the mean density of eosinophils in each GI segment. Afterwards, we divided the patients in three groups to access differences in the mean density of eosinophils between them. The first group included patients diagnosed with IBS, the second included patients diagnosed with functional dyspepsia (FD) and the third was the control group. The last group included patients with GI symptoms who underwent endoscopy but did not have a diagnosis of GI disease after the clinical work-up.

Clinical data such as age, gender, primary reason for endoscopy and the final diagnosis, were recorded and are presented in table 1.

This study was approved by the Ethic Committee for Health (CES) of Centro Hospitalar S.João, Porto, Portugal and Medical School of Porto, Portugal.

Tissue specimens

Biopsies from endoscopies of 33 patients performed between 2010 and 2017 at Centro Hospitalar S.João, Porto, Portugal were included in this study. Selected cases were subject to upper gastrointestinal endoscopy (n=15), lower gastrointestinal endoscopy

(n=15) or both (n=3). The endoscopic procedures included multiple biopsies of various segments of the GI mucosa (table 1). In 18 of the 33 patients, biopsies of the oesophagus, stomach and large intestine and in 28, biopsies of the small intestine were performed. Evaluated biopsies consisted of 33 specimens of oesophagus (proximal, mid and distal segment); 47 of stomach (fundus, body and antrum); 42 of small intestine (bulb, second segment of duodenum [SSD] and ileum); and 95 of large intestine (caecum, ascending, transverse, descending and sigmoid colon and rectum).

Selection of areas and counting

After selection of the patients to be included in this study, we retrieved the haematoxylin and eosin histology slides of each patient to count the eosinophils. These slides were reviewed together with another pathologist (P.C) to select the areas of interest for this study.

In each area, four images were taken at 400x magnification with each image having an equivalent representation of surface epithelium and lamina propria of the mucosa. Oesophagus specimens did not include *lamina propria* and were composed only of stratified squamous epithelium. Each image represented a HPF (400x) which included an area of 0.245 mm^2. Images were taken with the Olympus BX 43 microscope (camera Olympus DP73) to obtain high quality digital colour images of the areas to be counted. This assured consistency between pathologist's counting's.

Each image was used to count eosinophils; the entire image including the edges was used for counting. Counting was conducted manually with ImageJ software (National Institutes of Health) that counts the clicks on each eosinophil (Cell Counter plugin). Eosinophils were counted in two distinct locations: in the surface epithelium and in the *lamina propria*. Eosinophils were counted if there was an identifiable portion of the nucleus present along with associated granules. Eosinophils located in the basement membrane of the surface epithelium were considered as intraepithelial eosinophils. Eosinophils that were present within the epithelial layer of the crypt or within the crypt lumen were considered to be within the crypts. Eosinophils present in the mucosa that

were neither in the surface epithelium nor the crypts were considered within the *lamina propria*. Inflammatory cells within blood vessels, in Peyer's Patch or other large lymphoid aggregates were not quantified in this analysis. ImageJ was used by two pathologists, in a double-blind setting. Subsequently data were compared and reviewed by a senior pathologist (F.C). Representative histologic features of tissue specimens from each segment of the GI tract are shown in figure 1.

After manual counting with ImageJ software, the number of eosinophils in each image was entered into an Excel spreadsheet. Density of eosinophils was reported as mean±standard deviation eosinophils per mm^2.

Statistical methods

The Excel file was converted into a statistical data analysis (SPSS) database. Mean, standard deviation, median, minimum and maximum counts were calculated for all evaluated segments using SPSS software (IBM® SPSS® Statistics, version 25). The results in each region are summarized in table 2. The peak count represents the highest density of eosinophils observed in each GI segment. Because all data were nonparametric, the Mann-Whitney test was conducted for comparisons between the three groups. A P value < 0.05 was considered to be statistically significant.

RESULTS

Patient characteristics:

A total of 33 paediatric patients (21 females and 12 males) were included in our study. The mean age was 14.6±3.4 years. The most common reason for endoscopy was dyspepsia (n=11). Some of the symptoms were observed together (diarrhoea and abdominal pain were both present in 3 patients). The most common final diagnosis was FD (n=10). In 12 clinical cases the final diagnosis report recorded in the database only excluded GI pathology as cause for the symptoms.

Oesophagus

Eosinophils were uniformly absent in the epithelium. Given the consistency of eosinophil counting's between the three segments evaluated, the results in oesophagus were considered as a single segment (n=33).

Stomach

Biopsies from the fundus (n=14) demonstrated a mean value of 0.7±0.9/mm² in the *lamina propria*. Only in half of the evaluated areas (n=7) eosinophils were observed. Eosinophilic density in the body (n=15) was 0.3±0.6/mm² in the *lamina propria*. Eosinophils were only present in 3 of the evaluated areas. The peak count was 3 and 2 eosinophils/mm², in the fundus and body, respectively. In the antrum (n=18) were 0.6±1.5/mm² in the *lamina propria*. The peak count was 6 eosinophils/mm² and eosinophils were not observed in 14 of the analysed areas. All the evaluated segments revealed consistent eosinophil counting. Additionally, eosinophils were uniformly absent in the surface epithelium of the three evaluated segments.

Small Intestine

Biopsies from the bulb (n=13) revealed 17.8±16.6/mm² in the *lamina propria* and a peak count of 49 eosinophils/mm². The number of eosinophils/mm² present in the surface epithelium was 0.9±1.9, with a peak count of 7. Eosinophilic density in the lamina propria of the SSD (n=13) was 14.2±11.8/mm², with 1.4±2.1/mm² in the surface epithelium. The peak count observed in this segment was 41/mm² in the *lamina propria* and 7/mm² in the surface epithelium. Evaluation of biopsies from the ileum (n=16) revealed 50.4±34.6/mm² with a peak count of 109/mm². Distribution of eosinophils in the ileum was noted to be patchy, with a range of 3 to 109/mm². Eosinophils in the surface epithelium ranged from 0 to 9/mm² with a mean value of 3.3±2.8/mm².

Large Intestine

The mean number and the peak count of eosinophils in the *lamina propria* of the caecum (n=16) were the highest amongst evaluated segments (50.8±32.8 and

123/mm², respectively). The eosinophil counts were uniformly lower in the *lamina propria* of the other evaluated segments of the colon.

In the ascending colon (n=16) we observed a mean value of 40.1±26.8/mm² with a peak count of 86/mm². In the transverse colon (n=14) the eosinophil counting revealed 33.6±21.5/mm² with a peak count of 68/mm². In the descending colon (n=15), 39.2±26.1/mm² and a peak count of 90/mm². In the sigmoid colon (n=17), 25.3±17.4/mm² with a peak count of 55/mm². Finally, evaluated biopsies from the rectum (n=17) revealed 13.6±9.9/mm² and a peak count of 43/mm². Distribution of eosinophils in the large intestine was noted to be patchy as observed in the ileum. In the caecum, eosinophil counting ranged from 2 to 123/mm². In the other GI segments, we observed a similar amplitude of the eosinophil counting, with a lower peak count. Eosinophils, as observed in the upper GI tract, were regularly absent in the surface and crypt epithelium in these segments. Caecum was the segment with the highest eosinophilic density in the surface and crypt epithelium (4.1±3.7/mm²) and a peak count of 13/mm². Eosinophil numbers in the surface epithelium and crypt epithelium in the colon were: 2.9±3.0/mm² in the ascending; 2.9±3.0/mm² in the transverse; 2.9±2.6/mm² in the descending; 2.3±2.3/mm² in the sigmoid; and 1.8±2.3/mm² in the rectum.

Effect of IBS and FD in the number of eosinophils

FGID was the final diagnosis in 21 of the selected patients for this study. IBS was the final diagnosis in 6 patients. The symptoms of the 15 remaining patients were attributed to other FGID, namely FD (n=10), functional constipation (n=3), functional abdominal pain (n=1) and functional dysphagia (n=1). In order to compare the number of eosinophils between patients with FGID and no GI disease, we divided our population in 3 groups. We compared the mean density between the IBS and the control patients and between the FD and control patients, using the Mann Whitney test. In the IBS group we only had one biopsy specimen of stomach and small intestine (except ileum) so we were not able to make comparisons with the control group in these segments. The same occurred with

the ileum and large intestine segments in the FD group. Patients with other FGID were not included in this analysis. As eosinophils were uniformly absent in the oesophagus, this segment was not included in the comparison analysis. The number of eosinophils in the *lamina propria* was the only area compared between the groups. Table 3 shows the mean number of eosinophils in each group and *P* values of the comparisons made between the groups. The paired data did not reveal significant differences in the evaluated segments of the GI tract among the three groups. Notably, the patchiness in the distribution of eosinophils previously observed was maintained in all groups (figure 2).

DISCUSSION

EGID are a global growing concern which still faces limitations regarding its proper diagnosis. In most segments of the GI tract there is no consensus on specific limits for normality regarding the number of eosinophils. The scattered published data addressing the normal content of eosinophils includes three reports in children and four in adults.

As such, our goal was to determine the eosinophil content of the normal digestive mucosa in a paediatric population and to expand the scarce data available for each GI segment.

In the oesophagus we measured the content of eosinophils in the surface epithelium of the proximal, mid and distal segments. We did not differentiate each segment of the oesophagus as result of the absence of eosinophils in this particular area. These findings were consistent with other reports[8,11].

We also measured the mean and maximum number of eosinophils/HPF with Olympus BX43 to allow comparisons with publications reported in eosinophils/HPF. Although most of them had published their results in eosinophils/HPF[8,9], the most recent indications recommend that authors use eosinophils/mm^2. The mean and maximum

number/HPF were, respectively, 0.2 and 3 (fundus), 0.1 and 1 (body), 0.2 and 2 (antrum), 4.4 and 17 (bulb), 3.6 and 16 (SSD), 12.6 and 31 (ileum), 12.7 and 36 (caecum), 10.0 and 23 (ascending colon), 8.4 and 22 (transverse colon), 9.9 and 32 (descending colon), 6.3 and 18 (sigmoid colon) and 3.3 and 17 (rectum).

Contrary to published data[8,10,11], eosinophils were barely present in the segments of the stomach. In fact, most of the gastric biopsies were devoid of eosinophils. The highest peak count was reported in the fundus (3 eosinophils/HPF) whereas Debrosse *et al*[8] observed a peak count of 9 eosinophils/HPF in the same segment.

Our results indicate that every other GI segment has significant and variable number of eosinophils in the *lamina propria*. The range of eosinophil counting observed in our study group was consistent with most of the previous published data[8,9,11,12]. Generally, the number of eosinophils significantly increased from the oesophagus to the caecum and gradually decreased in the large intestine. However, in our report the eosinophilic density in the bulb was higher than in the SSD. To the best of our knowledge this is the first report evaluating biopsies from the bulb and SSD separately. Moreover, we observed a slight increase of the eosinophilic density and peak count in the descending colon. This pattern of distribution was different from previous studies. Other reports have differentiated segments in the large intestine, but not as many as this study. Until now, the majority of studies has shown a progressive gradient in the eosinophilic density from the oesophagus to the caecum and a gradual decrease along the large intestine[8,11]. In the report of Saad[9] there was another peak in the rectosigmoid segment. Both our study and the latest published results[8,9,11,12] showed consistently that a one-fits-all number may not be the best option for defining the limits of normality in the colon. The same may be applied to the small intestine where the normal content of eosinophils in the ileum is clearly superior to the segments of the duodenum.

We also evaluated the presence of eosinophils in the surface and crypt epithelium. Our results showed that eosinophils are primarily present in the *lamina propria* and not in the surface epithelium. These findings were consistent with previous published data[8].

We selected a paediatric population previously submitted to endoscopy that was reported as normal. It is important to note that endoscopic procedures, especially in paediatrics, have very strict criteria and indications. Therefore, our patients had GI symptoms and needed endoscopy to confirm or exclude GI pathology. In some of them the cause of the symptoms was not due to GI disease but remained undefined. We believe that exclusion of GI disease and normal associated histology of the mucosa were sufficient criteria to include these patients in our analysis.

Nowadays, in the presence of normal villous architecture and GI symptoms in children it is recommended to evaluate the number of duodenal intraepithelial lymphocytes as those may be increased in some diseases like CD[13,14]. Although we did not address the number of intraepithelial lymphocytes, the counts were normal at the time of clinical work-up.

It is increasingly apparent that eosinophils may play a pathogenic role in FGID[15,16]. Duodenal eosinophilia has been reported in FD[17]. IBS has also been linked to increased mast cells and other allergic-immune cells in the GI tract.[18] Regardless, we included patients with confirmed FGID. We evaluated the number of eosinophils separately in the IBS, FD and control patients. Despite small number of the cases, our results indicate that the number of eosinophils of FD and IBS patients was not statistically different than the number of eosinophils recorded in control patients. Moreover, the highest mean in the duodenum recorded in the FD group was 17.6±15.9 eosinophils/mm² whereas Wauters *et al*[17] reported a median of 151 eosinophils/mm². Patients diagnosed with other FGID were not compared because the role of eosinophils in those disorders is less studied. Because we evaluated more segments separately, our sample size in each segment was slightly inferior compared to other similar studies. However, our report revealed a consistent result that is comparable to previously published series.

We did not consider the seasonal variation in the number of eosinophils in our study, as reported by Polydorides *et al*[19]*.* Their data described an increase in the number of

eosinophils in colonic samples obtained between April and May. However, the relationship between allergen exposure and colonic eosinophilia was not significant. Another study concluded for no seasonal effect in the number of eosinophils[10].

We also dismissed the geographic variation even though it has been reported previously in Pascal *et al*[20]. Geographic variations in the number of eosinophils can be expected because allergy or parasitosis[21], recognizable causes of tissue eosinophilia, might differ between countries. Several studies were conducted in various regions of Portugal to access the prevalence of GI parasitosis between 1970 and 1990[22,23]. These studies showed a significant decrease in the prevalence of all GI parasites, due to the improvement in health care and generalized chemoprophylaxis of GI parasitosis. More recent studies reported comparable results[24,25]. We can assume that, currently, parasitosis in Portugal is not a significant cause of GI eosinophilia which could bias our results. Also, the low prevalence recorded in the various regions may allow generalization of our results.

In summary, these data provide a baseline count and distribution of eosinophils in the GI tract of paediatric patients with normal histology. Our analysis included patients with FGID who showed comparable results with the other patients. We believe that normal variations in the normal density of mucosal eosinophils should be first evaluated within a specific region. This is the first report addressing the normal distribution of eosinophils in an European population. Furthermore, we provide baseline values for GI segments which were not evaluated separately before, providing an additional contribution to evaluate children with suspected EGID.

REFERENCES

1. Cianferoni A, Spergel JM. Eosinophilic Esophagitis and Gastroenteritis. *Curr Allergy Asthma Rep*. 2015;15(9):58. doi:10.1007/s11882-015-0558-5.

2. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID)☆. *J Allergy Clin Immunol*. 2004;113(1):11-28. doi:10.1016/j.jaci.2003.10.047.

3. Furuta GT, Katzka DA. Eosinophilic Esophagitis. *N Engl J Med*. 2015;373(17):1640-1648. doi:10.1056/NEJMra1502863.

4. Collins MH. Histopathology Associated with Eosinophilic Gastrointestinal Diseases. *Immunol Allergy Clin North Am*. 2009;29(1):109-117. doi:10.1016/j.iac.2008.10.005.

5. Jung Y, Rothenberg ME. Roles and Regulation of Gastrointestinal Eosinophils in Immunity and Disease. *J Immunol*. 2014;193(3):999-1005. doi:10.4049/jimmunol.1400413.

6. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. *J Allergy Clin Immunol*. 2011;128(1):3-20. doi:10.1016/j.jaci.2011.02.040.

7. Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. *Mod Pathol*. 1996;9(2):110-114. http://www.ncbi.nlm.nih.gov/pubmed/8657715. Accessed December 22, 2017.

8. DeBrosse CW, Case JW, Putnam PE, Collins MH, Rothenberg ME. Quantity and Distribution of Eosinophils in the Gastrointestinal Tract of Children. *Pediatr Dev Pathol*. 2006;9(3):210-218. doi:10.2350/11-05-0130.1.

9. Saad AG. Normal Quantity and Distribution of Mast Cells and Eosinophils in the Pediatric Colon. *Pediatr Dev Pathol*. 2011;14(4):294-300. doi:10.2350/10-07-0878-OA.1.

10. Lwin T, Melton SD, Genta RM. Eosinophilic gastritis: histopathological characterization and quantification of the normal gastric eosinophil content. *Mod Pathol*. 2011;24(4):556-563. doi:10.1038/modpathol.2010.221.

11. Matsushita T, Maruyama R, Ishikawa N, et al. The Number and Distribution of Eosinophils in the Adult Human Gastrointestinal Tract. *Am J Surg Pathol*. 2015;39(4):521-527. doi:10.1097/PAS.0000000000000370.

12. Turner KO, Sinkre RA, Neumann WL, Genta RM. Primary Colonic Eosinophilia and Eosinophilic Colitis in Adults. *Am J Surg Pathol*. 2017;41(2):225-233. doi:10.1097/PAS.0000000000000760.

13. Shmidt E, Smyrk TC, Faubion WA, Oxentenko AS. Duodenal Intraepithelial Lymphocytosis With Normal Villous Architecture in Pediatric Patients. *J Pediatr Gastroenterol Nutr*. 2013;56(1):51-55. doi:10.1097/MPG.0b013e318267c353.

14. Hammer STG, Greenson JK. The Clinical Significance of Duodenal Lymphocytosis With Normal Villus Architecture. *Arch Pathol Lab Med*. 2013;137(9):1216-1219. doi:10.5858/arpa.2013-0261-RA.

15. Powell N, Walker MM, Talley NJ. Gastrointestinal eosinophils in health, disease and functional disorders. *Nat Rev Gastroenterol Hepatol*. 2010;7(3):146-156. doi:10.1038/nrgastro.2010.5.

16. Walker MM, Talley NJ. Functional Gastrointestinal Disorders and the Potential Role of Eosinophils. *Gastroenterol Clin North Am*. 2008;37(2):383-395. doi:10.1016/j.gtc.2008.02.007.

17. Wauters L, Nightingale S, Talley NJ, Sulaiman B, Walker MM. Functional dyspepsia is associated with duodenal eosinophilia in an Australian paediatric cohort. *Aliment Pharmacol Ther*. 2017;45(10):1358-1364. doi:10.1111/apt.14045.

18. Walker MM, Warwick A, Ung C, Talley NJ. The Role of Eosinophils and Mast Cells in Intestinal Functional Disease. *Curr Gastroenterol Rep*. 2011;13(4):323-330. doi:10.1007/s11894-011-0197-5.

19. Polydorides AD, Banner BF, Hannaway PJ, Yantiss RK. Evaluation of site-specific and seasonal variation in colonic mucosal eosinophils. *Hum Pathol*. 2008;39(6):832-836. doi:10.1016/j.humpath.2007.10.012.

20. Pascal RR, Gramlich TL, Parker KM, Gansler TS. Geographic variations in eosinophil concentration in normal colonic mucosa. *Mod Pathol*. 1997;10(4):363-365. http://www.ncbi.nlm.nih.gov/pubmed/9110299. Accessed December 22, 2017.

21. Mehta P, Furuta GT. Eosinophils in Gastrointestinal Disorders. *Immunol Allergy Clin North Am*. 2015;35(3):413-437. doi:10.1016/j.iac.2015.04.003.

22. Cruz AL da S. Parasitoses intestinais em crianças de idade escolar : Giardia lamblia: Ciclo de vida e sensibilidade a antiparasitários. 2003. https://repositorio-aberto.up.pt/handle/10216/10679. Accessed December 27, 2017.

23. Sociedade Portuguesa de Pediatria. S, Beorlegui M, Brito MJ, Rocha G. *Acta Pediátrica Portuguesa : Orgão Oficial Da Sociedade Portuguesa de Pediatria.* Vol 43. Sociedade Portuguesa de Pediatria; 2012. http://actapediatrica.spp.pt/article/view/639. Accessed December 27, 2017.

24. Lia Gata LG, Pereira MH, Tomé R, Salgado M. Parasitoses intestinais em crianças e adultos Estudos realizados em laboratórios do ambulatório e hospitalar. 2008. http://saudeinfantil.asic.pt/images/download-arquivo/2008 - 3 - Dezembro/rsi-2008-dezembro.pdf. Accessed December 27, 2017.

25. Sociedade Portuguesa de Pediatria. A, Costa JM, Valente CAP, Teixeira ME. *Acta Pediátrica Portuguesa : Orgão Oficial Da Sociedade Portuguesa de Pediatria.* Vol 35. Sociedade Portuguesa de Pediatria; 2004. http://actapediatrica.spp.pt/article/view/4977/3765. Accessed December 27, 2017.

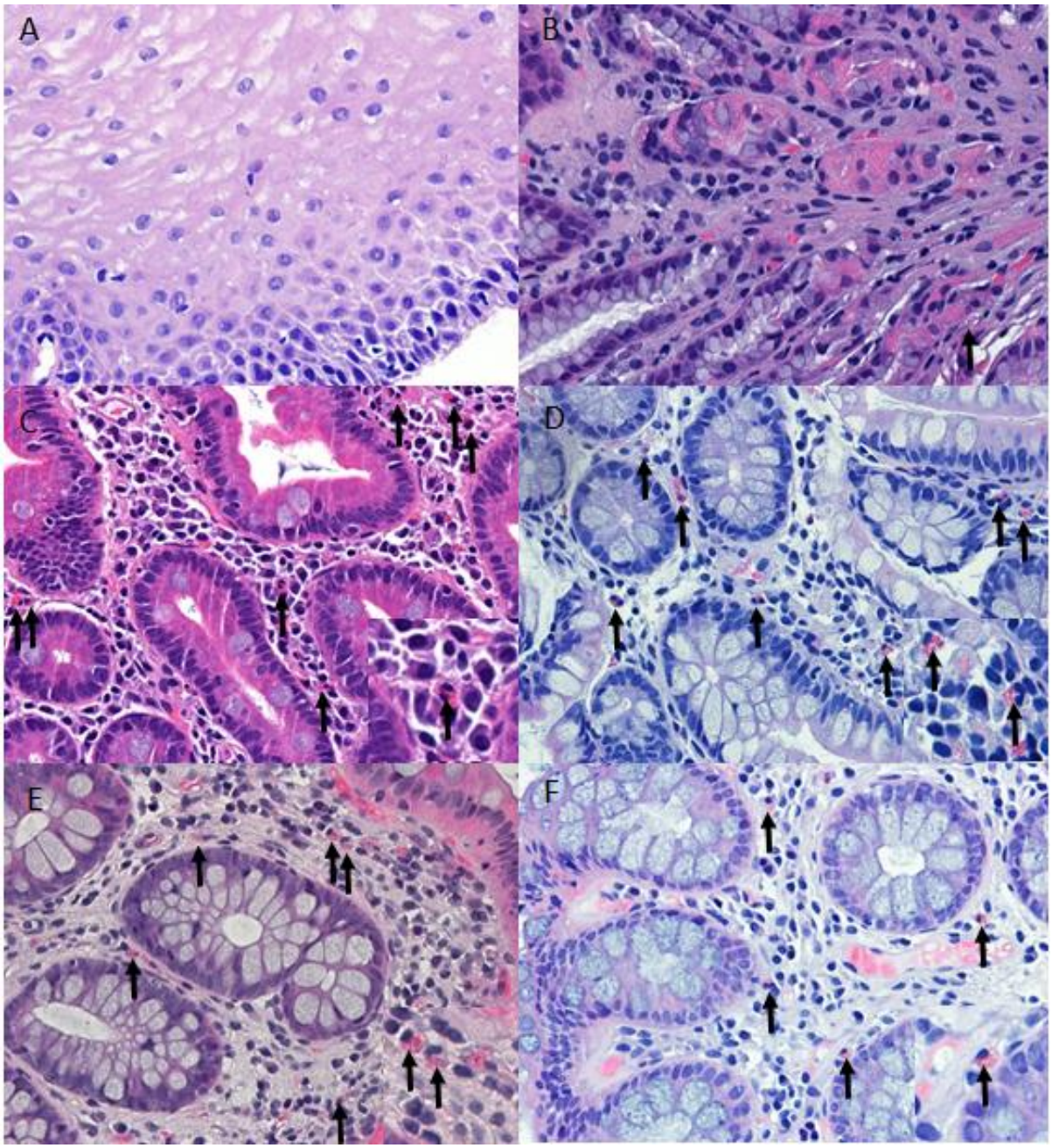
ANEXOS

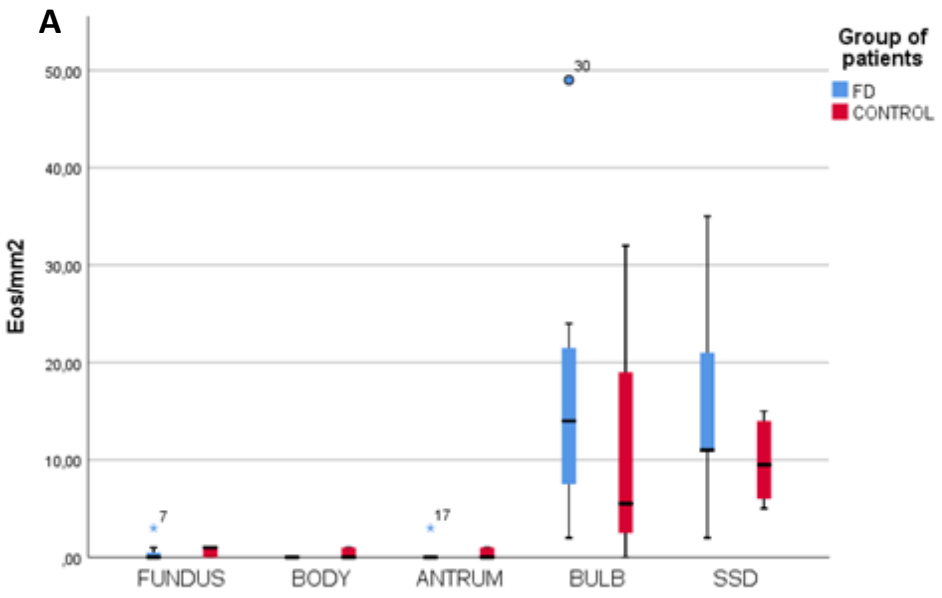
Figure 1 legend:

Photographies of histologic samples (haematoxylin and eosin) from biopsies of the gastrointestinal tract. A, Oesophageal epithelium. B, Gastric mucosa (Fundus). C, Duodenal mucosa (Bulb). D, Ileal mucosa. E, Descending colon mucosa. F, Rectal mucosa. The arrows indicate some of the eosinophils present in the lamina propria. Insets of B through F are high-power images clearly illustrating eosinophils.

Figure 2 legend:

Eosinophil levels in gastrointestinal segments of the various groups. A shows the differences in the distribution of eosinophils between the patients with functional dyspepsia and controls. B shows the differences in the distribution of eosinophils between the patients with Irritative Bowel Syndrome and controls. The solid black line represents the mean eosinophil level in the lamina propria of the respective segment. Abbreviations: FD, Functional Dyspepsia; IBS, Irritative Bowel Syndrome; SSD, second segment of duodenum; AC, ascending colon; TC, transverse colon; DC, descending colon; SC, sigmoid colon


A
B
C
D
E
F


A
Group of patients
FD
CONTROL
Eos/mm2
50,00
40,00
30,00
20,00
10,00
,00
30
7
17
FUNDUS
BODY
ANTRUM
BULB
SSD

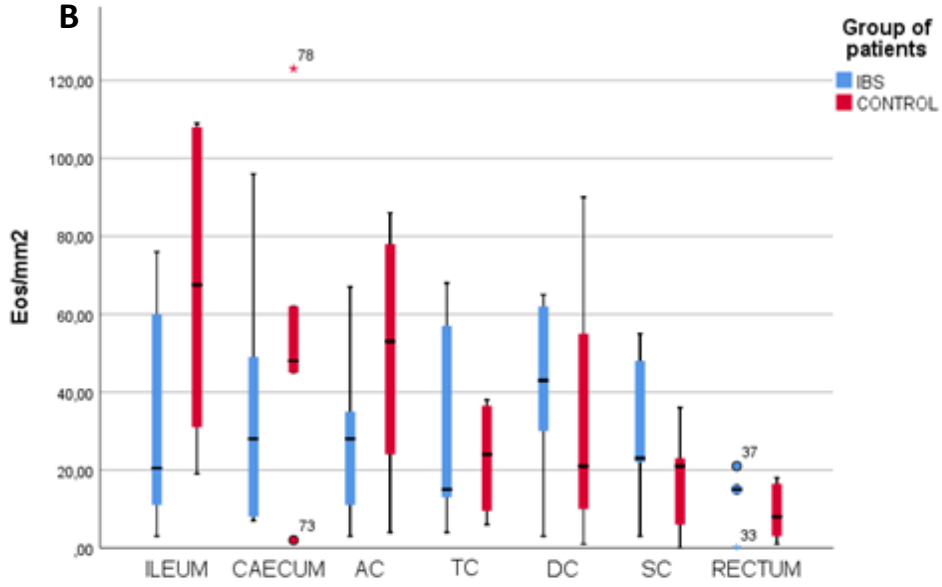

B
Group of patients
IBS
CONTROL
Eos/mm2
120,00
100,00
80,00
60,00
40,00
20,00
,00
78
73
37
33
ILEUM
CAECUM
AC
TC
DC
SC
RECTUM

TABLE 1:

Title: Clinical data of enrolled patients

Patient number	Age	Gender	Reason for UGE/LGE	Final diagnosis	Biopsy
1	18	F	Dyspepsia	Functional Dyspepsia	PE, ME, DE, F, BS, A, B
2	9	F	Recurrent emesis/Dyspepsia	Functional Dyspepsia	DE, A, BS, SSD
3	10	M	Dyspepsia	Functional Dyspepsia	DE, A
4	17	M	Dyspepsia	EGIP	PE, ME, DE, F, BS, A, B, SSD
5	16	M	Dyspepsia	Functional Dyspepsia	DE, F, BS, A
6	15	F	Enteropathy not confirmed	EGIP	PE, DE, F, BS, A, B, SSD
7	17	F	Recurrent emesis /Dyspepsia	Functional Dyspepsia	DE, F, BS, A, B, SSD
8	14	F	Dyspepsia	Functional Dyspepsia	DE, F, BS, A
9	12	F	Dyspepsia	Functional Dyspepsia	PE, ME, DE, F, BS, A, B, SSD
10	17	F	Recurrent emesis /Dyspepsia	EGIP	DE, F, BS, A, B, SSD
11	17	F	Dyspepsia	EGIP	PE, ME, DE, A, SSD
12	8	M	Dysphagia	Functional Dyspepsia	PE, ME, DE, F, BS, A, B, SSD
13	14	M	Dyspepsia	Functional Dyspepsia	ME, F, BS, A, B, SSD
14	8	M	Dysphagia	Functional Dysphagia	PE, ME, DE, F, BS, A, B, SSD
15	15	M	Dysphagia/AP	IBS	DE, F, BS, A, B, SSD, I, C, AC, TC, DC, SC, R
16	16	M	IDA	EGIP	I, C, AC, SC
17	17	F	Rectal bleeding/Diarrhoea	IBS	I, C, AC, TC, DC, SC, R
18	14	F	Diarrhoea/AP	EGIP	PE, DE, F, BS, A, B, SSD
19	14	F	Diarrhoea	IBS	SC, R
20	18	F	Recurrent emesis /AP	EGIP	I, C, AC, TC, DC, SC, R
21	16	F	Rectal bleeding/AP	IBS	I, C, AC, TC, DC, SC, R
22	17	M	Diarrhoea	IBS	DE, BS, A, B, I, C, AC, TC, DC, SC, R
23	18	F	AP	Functional Dyspepsia	I, AC, TC, DC, SC, R
24	18	F	Diarrhoea/AP	Functional AP	I, C, AC, TC, DC, SC, R
25	11	F	Diarrhoea/AP	EGIP	I, C, AC, TC, DC, SC, R
26	8	M	Rectal bleeding/Constipation	Functional Constipation	I, C, AC, TC, DC, SC, R
27	18	F	Diarrhoea	IBS	I, C, AC, TC, DC, SC, R
28	18	F	IDA/AP	EGIP	PE, DE, F, A, B, SSD, I, C, AC, S, R
29	14	F	IDA	EGIP	I, C, AC, TC, DC, SC, R
30	12	M	Rectal bleeding	EGIP	I, C, AC, DC, SC, R
31	11	M	Rectal bleeding	EGIP	I, C, AC, TC, DC, SC, R
32	18	F	Rectal bleeding/Constipation	Functional Constipation	I, C, TC, DC, SC, R
33	16	F	Constipation/AP	Functional Constipation	C, AC, TC, DC, SC, R

Legend: Patients characteristics are described, including age, gender (F: female; M: male), reason for endoscopy and the final diagnosis. Abbreviations: AP, abdominal pain; EGIP, exclusion of gastrointestinal pathology; IBS, Irritable Bowel Syndrome; IDA, iron deficiency anaemia; PE, proximal oesophagus; ME, medium oesophagus; DE: distal oesophagus; F, fundus; BS, body of stomach; A, antrum; B, bulb; SSD, second segment of duodenum; I, ileum; C, caecum; AC, ascending colon; TC, transverse colon; DC, descending colon; SC, sigmoid colon; R, rectum.

TABLE 2

Title: Eosinophilic density in the gastrointestinal tract

Gastrointestinal segment	Lamina propria			Surface or crypt epithelium		
	Mean	Median	Max	Mean	Median	Max
Oesophagus	N/A	N/A	N/A	0±0	0	0
Fundus	0.7±0.9	0.5	3	0±0	0	0
Body of stomach	0.3±0.6	0	2	0±0	0	0
Antrum	0.6±1.5	0	6	0±0	0	0
Bulb	17.8±16.6	14.0	49	0.9±1.9	0	7
Second segment of duodenum	14.2±11.8	11.0	41	1.4±2.1	1.0	7
Ileum	50.4±34.6	45.5	109	3.3±2.8	4.0	9
Caecum	50.8±32.8	49.0	123	4.1±3.7	3.0	13
Ascending colon	40.2±26.8	34.5	86	2.9±3.0	2.0	9
Transverse colon	33.6±21.5	36.5	68	2.9±3.0	2.0	11
Descending colon	39.2±26.1	43.0	90	2.9±2.6	2.0	10
Sigmoid colon	25.3±17.4	23.0	55	2.3±2.3	2.0	8
Rectum	13.6±9.9	15.0	43	1.8±2.3	1.0	9

Legend: The mean number (±standard deviation), median and maximum number of eosinophils per mm^2 for each anatomical region of the gastrointestinal tract and each region of the mucosa is shown. Abbreviations: N/A, not applicable.

TABLE 3

Title: Effect of functional gastrointestinal disorders on eosinophil number in the gastrointestinal tract (number/mm²)

Gastrointestinal segment	Mean ± standard deviation (maximum)			*P* Value	
	IBS	FD	Controls	IBS vs Controls	FD vs Controls
Fundus	N/A	0.6±1.1 (3)	0.6±0.5 (1)	N/A	0.459
Body of stomach	N/A	0±0 (0)	0.4±0.5 (1)	N/A	0.062
Antrum	N/A	0.3±0.9 (3)	0.3±0.5 (1)	N/A	0.339
Bulb	N/A	17.6±15.9 (49)	10.8±14.4 (32)	N/A	0.450
Second segment of duodenum	N/A	16.0±12.6 (35)	9.8±4.1 (15)	N/A	0.3602
Ileum	31.8±29.2 (76)	N/A	67.0±41.1 (109)	0.078	N/A
Caecum	37.6±36.9 (96)	N/A	54.7±39.2 (123)	0.522	N/A
Ascending colon	28.7±22.4 (67)	N/A	49.7±32.9 (86)	0.200	N/A
Transverse colon	31.4±29.0 (68)	N/A	23.0±15.9 (38)	0.712	N/A
Descending colon	40.6±25.4 (65)	N/A	35.4±36.7 (90)	0.602	N/A
Sigmoid colon	30.2±21.2 (55)	N/A	16.4±12.8 (36)	0.255	N/A
Rectum	13.2±7.9 (21)	N/A	9.4±7.5 (18)	0.624	N/A

Legend: The mean (±standard deviation) and maximum number of eosinophils calculated for each segment in the 3 groups is shown. No statistical differences were noted between the groups. Abbreviations: FD, functional dyspepsia; IBS, Irritable Bowel Syndrome; N/A, not applicable.

Guidelines and Instructions to Authors of the *Journal of Pediatric Gastroenteroly and Nutrition*

ETHICAL AND LEGAL CONSIDERATIONS
A submitted manuscript must be an original contribution not previously published (except as an abstract), must not be under consideration for publication elsewhere. Each person listed as an author is expected to have participated in the study to a significant extent.
Documented review and approval from a formally constituted review board (Institutional Review Board or Ethics committee) is required for all studies involving people, medical records, and human tissues, and for all animal studies.

Declaration of Funding Source
The conflict of interest disclosure and funding declaration must be included on the title page of the manuscript and in Editorial Manager.
Authors with nothing to declare should provide a statement to that effect. Manuscripts submitted without the required disclosures will be returned to the authors.

Conflicts of Interest
All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding

Title length: The manuscript title should have no more than 120 characters including spaces. Keywords for referencing should be included in the title. Please no abbreviations. Fancy or comical titles are inappropriate and will be asked to be revised. Trade names of drugs and other products must not appear in the article title.

Structured abstract and key words: Please refer to the table above for abstract requirements for various article types. Do not cite references in the abstract. Limit the use of abbreviations and acronyms. At first mention, please write out the full term for abbreviations (e.g. Celiac Disease (CD)). Use the following subheads in your structured abstract: Objectives, Methods, Results, and Conclusions. For Keywords, list three to five key words that are not included in the title.

Original Articles: Original research articles should be approximately 18 double-spaced, numbered pages, including the title page, references, figures, and tables. Failure to comply with length restrictions may result in a delay in the processing of your paper. The following length targets are recommended for Original Articles:

- Structured Abstract: maximum of 250 words
- Introduction: 1 page (about 250 words)
- Methods: 2-3 pages (up to 750-1000 words)
- Results: 2-3 pages (up to 750-1000 words)
- Discussion: 3-5 pages (up to 1000 words)
- References: limited to those critical and relevant to the manuscript (not more than 50)
- Tables and Figures: legends limited no more than 100 words each

What is Known/What is New: Immediately following the abstract all article types except where indicated in chart above, authors should include text for a summary box that will be published on the first page of all accepted articles. This text should highlight the significance of the article with the following guidelines in mind: What is known about this subject? What are the new findings and/or what is the impact on clinical practice? Use the format:

- What is known (2-4 bullet points listed beneath this heading)
- What is new (2-4 bullet points listed beneath this heading)

The total text should not exceed 100 words. As this section should be able to stand alone, at first mention of an abbreviation, please write out the full term.

Please use the following checklist to decrease the likelihood that your manuscript will be returned:

1. Title Page: Check for appropriate length and wording of the title. Title page should be on separate page from the abstract and manuscript.
2. Author and co-author information: Provide details for all of the authors/co-authors and corresponding author on the title page. The authors should be listed as: first name first, middle name second if applicable and family name last (bold surname/family name). Verify that co-author names, titles, affiliations and degrees are accurate and current.
3. On a separate page, list each author and his/her respective roles in the submitted work, documenting appropriate input for authorship.
4. Manuscript length and formatting: Check that the length of the manuscript and abstract do not exceed the maximum word count and are formatted as per instructions to authors for each. Abstract should be on a separate page from the title page and manuscript
5. Figures: Do not embed figures in manuscript. Cite the figures in the text of the manuscript. Provide figure legends on a separate page. Check that the figures and supplementary figures are formatted as indicated in instructions to authors. Each figure should be in a separate file (uploaded individually).
6. Tables: Do not embed tables in manuscript. Cite the tables in the text of the manuscript. Provide table legends. Check that the tables and supplementary tables are formatted as indicated in instructions to authors. Each table should be in a separate file (uploaded individually).
7. References: Make sure each reference is cited in the text, references are formatted per instructions to authors and the quantity does not exceed maximum for the article type.

Unidade de Investigação

Tomei conhecimento. Nada a opor.

20 de Janeiro de 2018

A Coordenadora da Unidade de Investigação

(Prof.ª Doutora Ana Azevedo)

DIRECÇÃO CLÍNICA

Aprovado. Ao CA. 23.1.18

(Prof.ª Doutora Ana Azevedo)

AUTORIZADO 238-17

CONSELHO DE ADMINISTRAÇÃO REUNIÃO DE 25 JAN 2018

Presidente do Conselho de Administração

(Dr. António Oliveira e Silva)

Diretor Clínico | Enfermeira Diretora | Vogal Executivo | Vogal Executivo

(Prof. Dr. José Artur Paiva) | (Enf.ª Filomena Euridice) | (Dr. Luís Porto Gomes) | (Dr. Alberto G. Matos)

Exmo. Senhor

Presidente do Conselho de Administração do

Centro Hospitalar de S. João – EPE

Assunto: Pedido de autorização para realização de estudo/projecto de investigação

Nome do Investigador Principal: Jorge Manuel Ramos das Neves da Silva

Título do projecto de investigação: Quantificação de eosinófilos na mucosa digestiva em população pediátrica sem patologia orgânica.

Pretendendo realizar no(s) Serviço(s) de Pediatria e Anatomia Patológica do CH S.João. do Centro Hospitalar de S. João – EPE o estudo/projecto de investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efectivação.

Para o efeito, anexa toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de S. João respeitante a estudos/projectos de investigação, à qual endereçou pedido de apreciação e parecer.

Com os melhores cumprimentos.

Porto, 26 / Setembro / 2017

O INVESTIGADOR/PROMOTOR

Comissão de Ética para a Saúde do C.H.S.João e da FMUP

Parecer

Título do Projecto: Quantificação de eosinófilos na mucosa digestiva em população pediátrica sem patologia orgânica

Nome do Investigador Principal: Jorge Manuel Ramos Neves da Silva

Promotor do Estudo: NA

Serviço onde decorrerá o Estudo: Serviço de Pediatria e Serviço de Anatomia Patológica do Centro Hospitalar de S. João

Objectivo e Pertinência do Estudo: Trata-se de um trabalho que visa a determinação e quantificação do número de eosinófilos na mucosa digestiva normal, numa população pediátrica submetida a procedimentos endoscópicos, devidamente protocolados, sem patologia orgânica identificada.

O acesso aos dados do processo clínico será mediado pelo Dr Jorge Amil Dias

A Senhora Directora do Serviço de Neonatalogia deu o seu aval à realização deste projecto.

Benefício/risco: NA, dada a natureza retrospectiva do estudo.

Respeito pela liberdade e autonomia do sujeito de ensaio: NA

Confidencialidade dos dados: Os dados serão codificados, sem registo do nome, do número do processo clínico e do centro de seguimento.

Profissional de ligação: Dr Jorge Amil Dias (Serviço de Pediatria) e Profª Doutora Fátima Carneiro (Serviço de Anatomia Patológica)

Indemnização por danos: NA

Continuação do tratamento: NA

Propriedade dos dados: Trata-se de uma Tese de Mestrado da FMUP, prevendo-se a sua divulgação nos meios científicos.

***Curriculum* do investigador**: Adequado

Data previsível da conclusão do estudo: Janeiro 2018

Conclusão: Dada a natureza da investigação e os seus objectivos, proponho à CES um parecer favorável á sua realização.

Porto e C.H.S.João, 2017-10-13

O Relator

Doutor Filipe Almeida

7. SEGURO

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)

NÃO ☐

NÃO APLICÁVEL ☑

8. TERMO DE RESPONSABILIDADE

Eu, Jorge Manuel Ramos das Neves da Silva,

abaixo-assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo no decurso do actual internamento ou da mesma consulta.

Porto, 26 / Setembro / 2017

O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO

emitido na reunião plenária da CES de 13 / Setembro / 2017

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.